I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450, ON THE DATE INDICATED BELOW.

By: DEBRA DUNN-BROWN Date: 1/12/07

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of: Stephen Brian Falder *et al.*		
		Group Art Unit: 1616
Appln. No.:	10/039,677	Examiner: Alton N. Pryor
Filing Date:	January 4, 2002	Attorney Docket No.: 16644/09005CIP (BYOCX/P25765US)

Title: ANTI-MICROBIAL COMPOSITION

DECLARATION OF UNDER 37 C.F.R. § 1.132

I, Ulrich W Schwarz, declare as follows:

1. I am a German citizen residing in Germany. I have previously been engaged by Byotrol PLC as a consultant in relation to the subject matter of the present application and I am now an employee of Byotrol PLC. I understand that Byotrol PLC is the assignee of the above-identified application.

2. My professional experience includes Head of Clinical Lab (1979 – 1981), Medical Director Pharmaceutical Company (1982 – 1984), Scientific Adviser Drägerwerk AG in Industrial & Environmental Hygiene (1985 – 1992) (This special working area included microbial testing indoors (mould testing), testing of hygienic conditions in hospitals, food industry and other workplaces.), Consultant in Food & Environmental Hygiene (1992 – 1996) (This included microbial testing in food industry, surface testing, raw material testing and personal testing), Technical Director Medical Company developing *in vitro* Diagnostics (Sandwich Immuno Assay: hormone tests, drug of abuse tests, infections disease tests) and Application of EN & ISO Standards & Quality Management Systems (1997 – 2004), Consultant to medical companies in Shanghai & Hangzhou PRC in relation to the

Application of Quality Management Systems (2004), Consultant in nanotechnology (modification of surfaces on nano level: food industry, metal processing industry, glass industry) (2005).

3. I hold degrees of Bachelor of Science and Masters in Chemistry from Westf. Wilhelms Universitat Munster and DoctorNatrural Science (Dr. rer. nat.) from Technical University Darmstadt. Thesis: Conformational changes of tRNA during protein biosynthesis.

4. Some of my work in relation to the subject matter of this invention has been conducted in association with Professor Wolfgang Hillen of Friedrich-Alexander University, Erlangen, Germany. Professor Hillen is a world-renowned expert in the field of Microbiology. A copy of Professor Hillen's curriculum vitae is attached.

5. I have read and understood US Patent Application No. 10/039,677 (which I understand was published as US2003/0031687), the Office Action dated 12 July 2006 and the prior art documents to which the Examiner has referred. I am familiar with the amended claims currently under consideration.

6. I understand that the examiner considers that the claimed subject matter would have been obvious over Jackson (GB-A-2247171) in view of Dorothy (GB-A-2338651).

7. I have been responsible for conducting a number of experiments to illustrate the surprising and unexpected properties of the compositions of the invention. I have also studied experimental reports of other experiments that have been conducted to illustrate the advantages of the invention.

8. A summary of some of the experiments conducted and the results obtained is provided in attached Annexes I and II.

9. The attached Annexes are:

Annex I Report of Experiments Conducted by Dr Schwarz and Prof Hillen to Illustrate the Characteristic Properties of the Compositions which are the subject of US Patent Application No. 10/039,677.

Annex II Report of Experiments Conducted to Illustrate the Residual Effect of the Compositions which are the subject of US Patent Application No. 10/039,677.

10. Annex I describes an experiment that was conducted to illustrate the residual antimicrobial effect of compositions of the invention (see pages 5 to 7). The composition of the present invention was applied to a bathroom surface in two passenger cabins in a cruiser liner. The surface was then cleaned on a daily basis using water only. The total microbial count on the surface was tested on the third and the seventh day after application of the composition. The results reported show that the compositions of the invention provided a residual antimicrobial effect, largely reducing or controlling the formation of colonies of microorganisms on a surface for up to a week (the limit of this experiment) after application of the composition even when that surface is washed daily with water.

11. Annex II also describes some experiments that illustrate the residual antimicrobial effect of the compositions of the invention. A composition of the invention was applied to a surface and allowed to dry on that surface. When the surface was dry, a protein solution was applied to the surface and was dried. The surface was then rinsed extensively with distilled water. The water rinsing removed most of the protein solution from the surface, but did not remove the inventive composition from the surface. A comparative test was also carried out in which the same procedure was followed except that an anti-microbial composition that did not comprise a polysiloxane was used in place of the composition of the invention. In this comparative test rinsing extensively with distilled water did not remove the protein layer. These results indicate that a microbial biofilm would be unable to adhere to a surface treated with the composition of the present invention while it would be able to adhere to a surface treated with the comparative anti-microbial composition. This experiment illustrates the residual effect of the inventive composition in that in addition to killing microbes present at a surface at the time a surface is treated with the composition, further microbial biofilm formation at that surface is also prevented.

12. Annex I also describes a number of experiments that were conducted to illustrate the surprising and unexpected antimicrobial effect that is achieved by the combination of a quaternary ammonium compound and a low surface tension material, as defined in the claims of the present application, as compared to a quaternary ammonium compound in the absence of a low surface tension material (similar to Jackson).

13. In one of the experiments described in Annex I, cotton cloths were soaked in three compositions or deionised water (control). The three compositions were a solution of low surface tension material such as Clearco (a polysiloxane, similar to Dorothy), a solution of a quaternary ammonium compound and a solution of Clearco and the quaternary ammonium compound. The treated cloths were stored at room temperature until they were completely dry. Each of the dry pre-treated cloths and the control cloths were placed into Petri dishes filled with 5 mL of deionised water and let stand for 5 minutes. The washed cloths were then dried. Each dry, washed cloth was challenged with 300 μL E. Coli and allowed to completely dry at room temperature. When dry, the cloths were incubated at 37°C for 2 hours. Deionised water was then added to each Petri dish to elute surviving E. coli. The plates were evaluated for viable colony forming units.

14. As can be seen from the report, the low surface tension materials (which are similar to Dorothy) do not themselves have any antimicrobial activity. The quaternary ammonium compounds (which are similar to Jackson) were effective only at concentrations of 1.5% and 0.07%, but not at 0.025%. However, when a low surface tension material is used in combination with a quaternary ammonium compound, the antimicrobial properties of the resulting composition are unexpectedly enhanced. In fact, when the low surface tension material was used in combination with the quaternary ammonium compound as claimed in the present invention, the composition was effective as an anti-microbial agent at concentrations of 2.5%, 1.5%, 0.125%, 0.07%, 0.042%, and even 0.025%. These results were confirmed in a second experiment which tested the compositions over a broader concentration range.

15. The composition of the present invention was unexpectedly more effective as an anti-bacterial agent than the either of the test compositions. It is especially surprising that a low surface tension material, which does not have any antimicrobial effect alone, would enhance the antimicrobial effect of the quaternary ammonium compound.

16. The results of these experiments illustrate that both the residual effect of the compositions of the invention and the enhanced antimicrobial effect that is achieved by the combination of a quaternary ammonium compound and a low surface tension material were surprising and unexpected. One could not have predicted that the combination of a quaternary ammonium compound and a low surface tension material was much more effective than either composition alone. This is especially surprising based on the fact that the low surface tension material was wholly ineffective as an antimicrobial composition. Similarly, one could not have predicted that the combination of a quaternary ammonium compound and a low surface tension material would have a residual effect on the growth of microbial colonies.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present application or any patent issued thereon.

Signed: Ulrich W. Schwarz

This 08 day of January 2007

ANNEX I

REPORT OF EXPERIMENTS CONDUCTED BY DR SCHWARZ AND PROF HILLEN TO ILLUSTRATE THE CHARACTERISTIC PROPERTIES OF THE COMPOSITIONS WHICH ARE THE SUBJECT OF US PATENT APPLICATION NO. 10/039,677 ASSIGNED TO BYOTROL PLC

Materials 2
LB Medium (Agar Medium) 2
Quaternary Ammonium Compound: BAC 2
Low Surface Tension Compounds 2
Byotrol Formulation 3
CADDYCARE 3
Demonstration of Residual Effect 4
Test to Show the Effect of the Combination of a Quaternary Ammonium Compound and a Low Surface Tension Material 7
Testing "additive effect" of polymer at limited biocide concentrations: Cloth Samples (1) 7
Testing "additive effect" of polymer at limited biocide concentrations: Cloth Samples (2) 9
Testing "additive effect" of polymer at limited biocide concentrations: 11
Pretreatment Of Agar Plates 11
Testing "additive effect" of different polymers at limited biocide concentrations: 13
Pretreatment Of Agar Plates 13

MATERIALS

The following materials were used in the experiments that are described in this report.

LB Medium (Agar Medium)

LB Medium was used as buffer and liquid nutrient broth. This medium contained:

- 10 g/l Tryptone
- 5 g/l Yeast extract
- 10 g/l Sodium chloride
- pH was adjusted to 7

LB Agar plates containing this medium were also used.

Quaternary Ammonium Compound: BAC

A solution comprising:

CAS No	Compound	Concentration
61789-71-7	Coco alkyl dimethylbenzyl ammonium chloride	12.5 %

Was used as an example of the first anti-microbial agent as defined in currently pending claim 1 of US Patent Application No. 10/039,677. This solution was an aqueous solution of the quaternary ammonium compound and was obtained from Thor Ltd. The solution was diluted with distilled water as necessary to give the concentration used in the experiments reported here in.

Low Surface Tension Compounds

The following materials were used as representative of the at least one compound having a low surface tension as defined in currently pending claim 1 of US Patent Application No. 10/039,677.

ACTIVE SILICONE

CAS No	Compound	Concentration
63148-62-9	Polydimethylsiloxane	2.5 %
	As stabilizer: Ethoxylated Nonyl Phenol [9016-45-9]	

CLEARCO

CAS No	Compound	Concentration
541-02-6	Decamethylcyclopentasiloxane	2.5 %
	As stabilizer: Ethanol 75 %	

PS034

CAS No	Compound	Concentration
107460	Hexamethyldisiloxane As stabilizer: Ethanol 75 %	2.5 %

All of these materials were obtained from Clearco.

Byotrol Formulation

As an example of a formulation of the invention containing the components as defined in currently pending claim 1 of US Patent Application No. 10/039,677 and at least one additional anti-microbial agent the following formulation was used.

A1616 PRODUCT (F4L Ready To Use)

CAS-No.	COMPOUND	Active Conc.
61789-71-7	coco alkyl dimethylbenzyl ammonium chloride	0.07 %
7173-51-7	di-n-decyl dimethylammonium chloride	0.07 %
52-51-7	bronopol (INN)	0,05 %
27083-27-8	Polymeric Biguanide Hydrochloride	0,03 %
64-17-5	ethanol	0,13 %
990001-58-01	polydol	0,015 %
	water	99,64 %

This formulation will be referred to herein after as "the Byotrol formulation".

SUPERNOVA WIPES

Disposable wipes impregnated with the A1616 product (F4L Ready To Use). These wipes were taken from a tub containing approximately 150 wipes and approximately 700 ml of the Byotrol solution.

CADDYCARE

Caddycare is one brand name for the Byotrol A1616 F4L formulation described above. In these experiments it was used in spray form.

DEMONSTRATION OF RESIDUAL EFFECT

A test to demonstrate the residual antimicrobial effect of compositions of the invention was conducted in the bathroom of two passenger cabins of a cruise liner.

A bathroom was checked for total microbial counts prior to cleaning. This was done using a commercial surface test (Environcheck, Merck). Three different areas of the bathroom were checked as shown in Figure 1. The same areas of each bathroom were tested in each of the tests carried out in the rest of this experiment.

The results for the uncleaned bathroom are shown in Figure 1 (Cabin A).

The bathrooms were then cleaned using standard methods. By this we mean that they were cleaned with water and a disinfecting cleaner (containing didecyldimethyl ammonium chloride and ethoxylated nonyl phenol)

The bathrooms were checked again for total microbial counts. The results are in Figure 1 (Cabins B and C).

From Figure 1 it can be seen that cleaning with the disinfecting cleaner and water was ineffective in killing micro-organisms present on the surfaces tested.

The bathrooms were then treated by spraying with the Byotrol formulation.

Daily cleaning of the two bathrooms was then carried out using water only. The total microbial count was checked on the third day and on the seventh day. The results are shown Figure 2.

These results clearly show that the compositions of the invention provide a residual effect in that they prevent growth of micro-organisms at a surface that they have been used to treat even when that surface has been subjected to repeated washing.

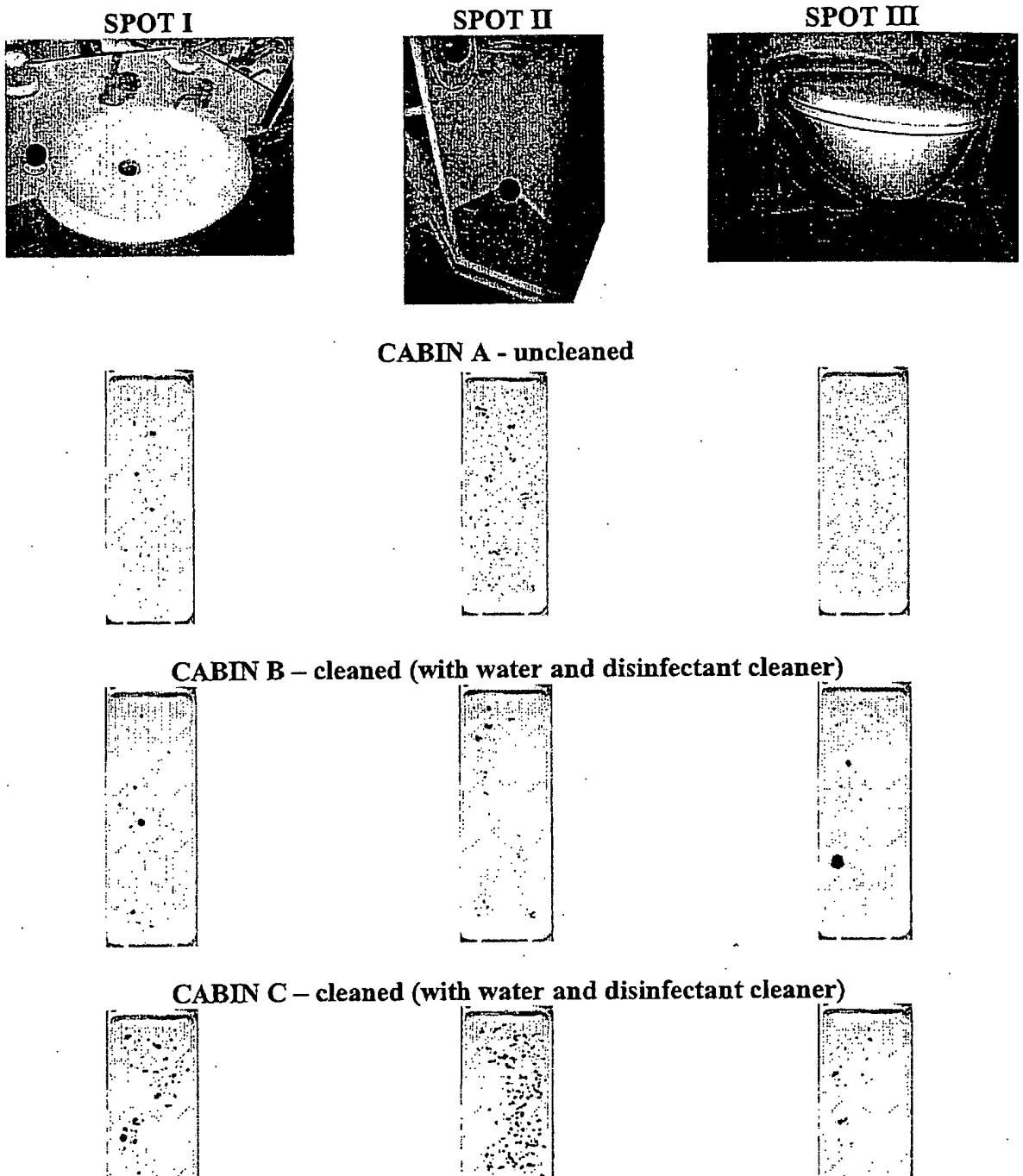
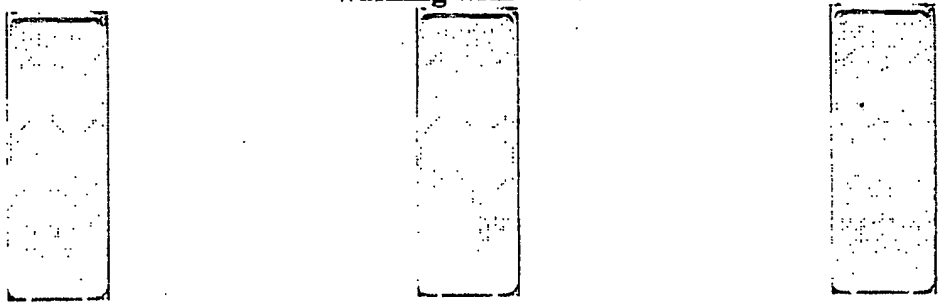

SPOT I
SPOT II
SPOT III
CABIN A - uncleaned
CABIN B – cleaned (with water and disinfectant cleaner)
CABIN C – cleaned (with water and disinfectant cleaner)

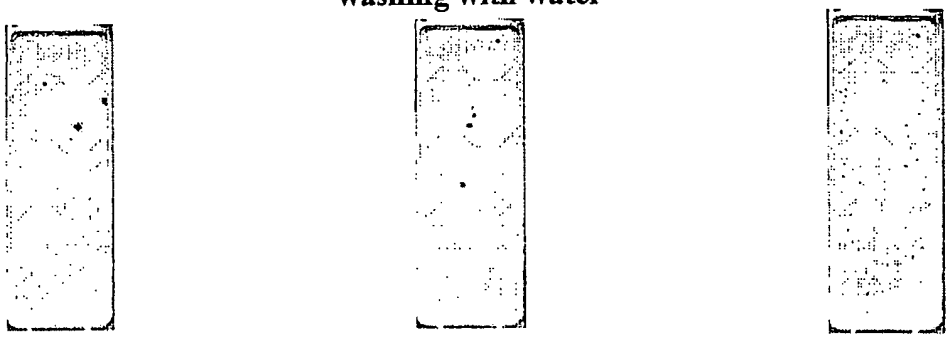
FIGURE 1

SPOT I SPOT II SPOT III

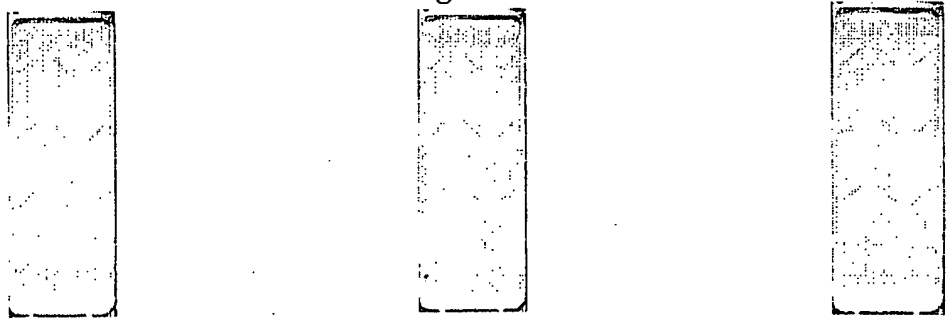
CABIN B – 3 days after treatment with the A1616 F4L composition and after daily washing with water



CABIN B – 7 days after treatment with the A1616 F4L composition and after daily washing with water



CABIN C – 3 days after treatment with the A1616 F4L composition and after daily washing with water



CABIN C – 7 days after treatment with the A1616 F4L composition and after daily washing with water

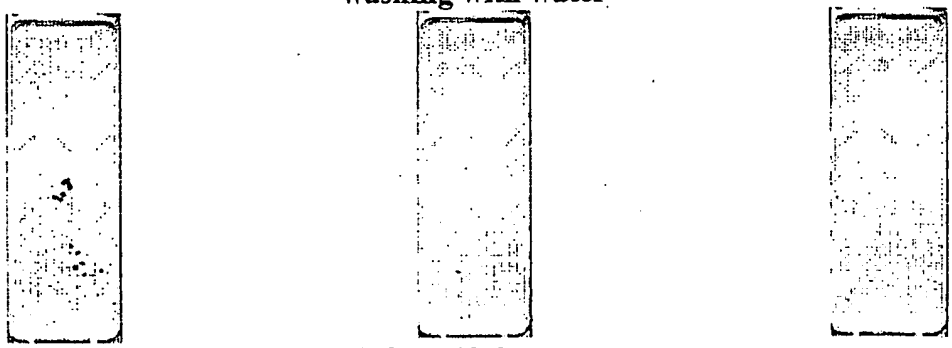


FIGURE 2

TEST TO SHOW THE EFFECT OF THE COMBINATION OF A QUATERNARY AMMONIUM COMPOUND AND A LOW SURFACE TENSION MATERIAL

Testing "additive effect" of polymer at limited biocide concentrations: Cloth Samples (1)

Testing of "additive effect" was done at limited (very low) biocide concentrations.

MATERIALS

The following materials were used:

Cloth (Cotton, 2x2 cm), LB Medium, BAC, CLEARCO, Wipe (Supernova soaked wipe), LB Agar plates, Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium (OD_{600}: 7.8 / mL (Reference: LB Medium) ≈ 7.8 x 10^9 cells/mL), deionised water.

Test liquids

#	BAC	CLEARCO	BAC + CLEARCO	
			BAC	CLEARCO
1	1.5 %	2.5 %	1.5 %	2.5 %
2	0.07 %	0.125 %	0.07 %	0.125 %
3	0.025 %	0.042 %	0.025 %	0.042 %

TESTING PROCEDURE

Pre-treatment of cloths
The cloths were soaked with test liquids (as shown in the above table) or deionised water (control 1) and stored at room temperature till they were completely dry (about 1 hr). Control 2 (Supernova wipe) was also stored at room temperature.

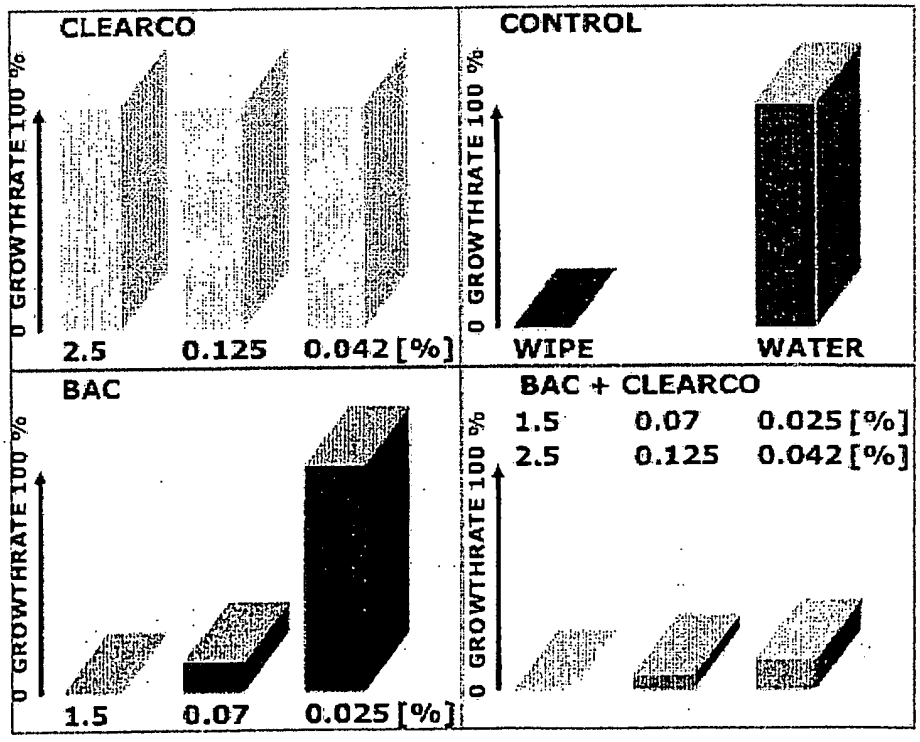
Washing of pre-treated clothes
Each of the dry pre-treated cloths and the control cloths were placed into a plastic Petri dish. The Petri dishes were filled with 5 mL of deionised water and left to stand for 5 minutes. The washed cloths were stored at room temperature till they were completely dry (about 1 hr).

Challenge with E. coli
Each dry pre-treated, washed cloth and each control cloths was challenged with 300 µL E. coli (10^6 cells in LB Medium) and allowed to completely dry at room temperature (about 30 minutes). When completely dry, the cloths were placed on LB Agar Petri dishes and incubated at 37 °C for 2 hrs. 200 µL deionised water was added to each Petri dish to elute surviving E. coli.

Incubation
The eluted E. coli samples were transferred to LB Agar plates and incubated at 37 °C for 48 hrs.

Evaluation
The plates were then evaluated for viable colony forming units (CFUs) such that 100% corresponds to an uncountable number of CFUs and 10% corresponds to approximately 10^2 CFUs. The results are presented graphically below.

Results and Interpretation
As shown in the graphs below, under the test conditions described above BAC alone was active as an anti-microbial agent at concentrations of 1.5 % and 0.07 % but it was ineffective at a concentration of 0.025 %. The polymer CLEARCO showed no antimicrobial activity when used alone. The combination of BAC and CLEARCO showed strong antimicrobial activity at all the concentrations tested. Particularly significant is the fact that the combination of BAC and Clearco having a BAC concentration of 0.025% had much stronger antimicrobial activity that this concentration of BAC in the absence of the low surface tension material Clearco. The general activity of the test design was cross checked by using water as control 1 (no antimicrobial activity) and the Supernova Wipe (strong antimicrobial activity) as control 2.



Testing "additive effect" of polymer at limited biocide concentrations: Cloth Samples (2)

A test similar to that described above was carried out using BAC and CLEARCO over a large concentration range. Again, testing of "additive effect" was done at limited (very low) biocide concentrations (including concentrations lower than those used in the test described above).

MATERIALS

Cloth (Cotton, 2x2 cm), LB Medium, BAC, CLEARCO, LB Agar plates, Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium (OD_{600}: 3.0 / mL (Reference: LB Medium) ≙ 3.0 x 10^9 cells/mL), deionised water.

Test liquids

#	BAC	CLEARCO	BAC + CLEARCO	
			BAC	CLEARCO
1	1.5 %	2.5 %	1.5 %	2.5 %
2	0.07 %	0.125 %	0.07 %	0.125 %
3	0.051 %	0.098 %	0.051 %	0.098 %
4	0.038 %	0.064 %	0.038 %	0.064 %
5	0.031 %	0.051 %	0.031 %	0.051 %
6	0.025 %	0.042 %	0.025 %	0.042 %
7	0.021 %	0.036 %	0.021 %	0.036 %
8	0.019 %	0.031 %	0.019 %	0.031 %
9	0.017 %	0.028 %	0.017 %	0.028 %
10	0.015 %	0.025 %	0.015 %	0.025 %
11	0.013 %	0.023 %	0.013 %	0.023 %

TESTING PROCEDURES

Pre-treatment of cloths
The cloths were soaked with test liquids (as shown in the above table) or deionised water (control 1) and stored at room temperature till they were completely dry (about 1 hr).

Washing of pre-treated clothes
Each of the dry pre-treated cloths and the control cloths were placed into a plastic Petri dish. The Petri dishes were filled with 5 mL of deionised water and left to stand for 5 minutes. The washed cloths were stored at room temperature till they were completely dry (about 1 hr).

Challenge with E. coli

Each dry pre-treated, washed cloth and each control cloths was challenged with 100 µL E. coli (3 x 10^4 cells in LB Medium) and allowed to completely dry at room temperature (about 30 minutes). When completely dry, the cloths were placed in 1 mL LB Medium for extraction of E. coli (about 10 minutes). 100 µL of extraction buffer were diluted with 900 µL LB Medium. 300 µL of this dilution were placed on Agar Plates. 300 µL should contain ~ 10^3 E. coli.

Incubation

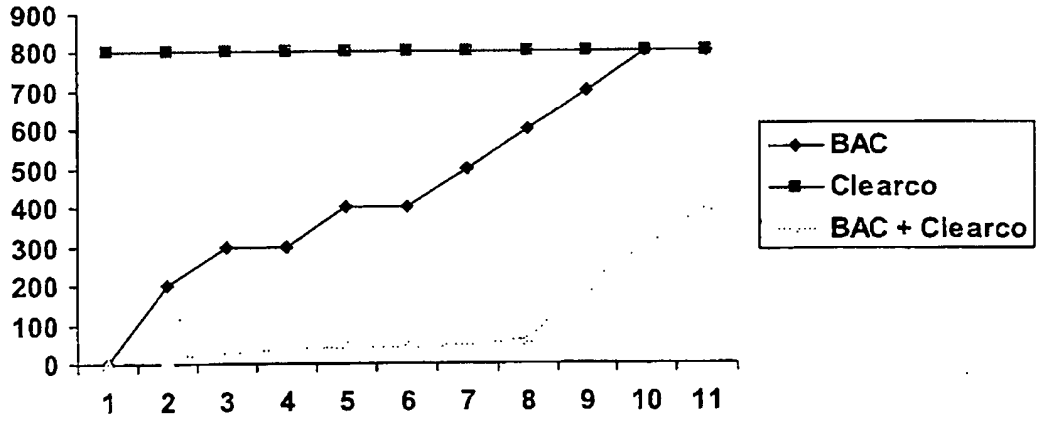
The E. coli samples were incubated at 37 °C for 48 hrs.

Evaluation

A grid method was used to count the number of CFUs in a given area. The results are presented graphically below (where the numbers on the X axis represent the test liquid number in the Table above and the numbers on the Y axis represent the number of CFUs in a square of the grid).

Results and Interpretation

As shown in the graph below, under the test conditions described above BAC alone was active as an anti-microbial agent at the higher concentrations used but as the concentration decreased BAC alone became rapidly less effective and it was ineffective at lower concentrations. The polymer CLEARCO showed no antimicrobial activity when used alone. The combination of BAC and CLEARCO showed strong antimicrobial activity at even at lower concentrations of BAC. The general activity of the test design was cross checked by using water as control 1 (no antimicrobial activity).



Testing "additive effect" of polymer at limited biocide concentrations: Pretreatment Of Agar Plates

Testing of "additive effect" was done at limited (very low) biocide concentrations.

MATERIALS

LB Agar plates, LB Medium, BAC, CLEARCO, Caddycare (contains A1616 F4L Ready To Use), Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium (OD_{600}: 4.3 / mL (Reference: LB Medium) ≙ 4.3 x 10^9 cells/mL), deionised water.

Test liquids

#	BAC	CLEARCO	BAC + CLEARCO	
			BAC	CLEARCO
1	1.5 %	2.5 %	1.5 %	2.5 %
2	0.07 %	0.125 %	0.07 %	0.125 %
3	0.025 %	0.042 %	0.025 %	0.042 %

TESTING PROCEDURES

Pre-treatment of agar plates

1 mL of the test solutions (as shown in the above Table) was added to LB Agar plates and spread of the surface of the plates as a film. As control 1, 1 ml of deionised water was added to a LB Agar plate and spread of the surface of the plate as a film. As control 2, 1 ml of Caddycare was added to a LB Agar plate and spread of the surface of the plate as a film. The treated LB Agar plates were stored at room temperature for 6 hrs to allow the test solutions to diffuse into the agar.

Challenge with E. coli

Each pre-treated LB Agar plate was challenged with 300 µL E. coli (10^6 cells in LB Medium) covering the agar surface.

Incubation

The Agar plates were incubated at 37 °C for 48 hrs.

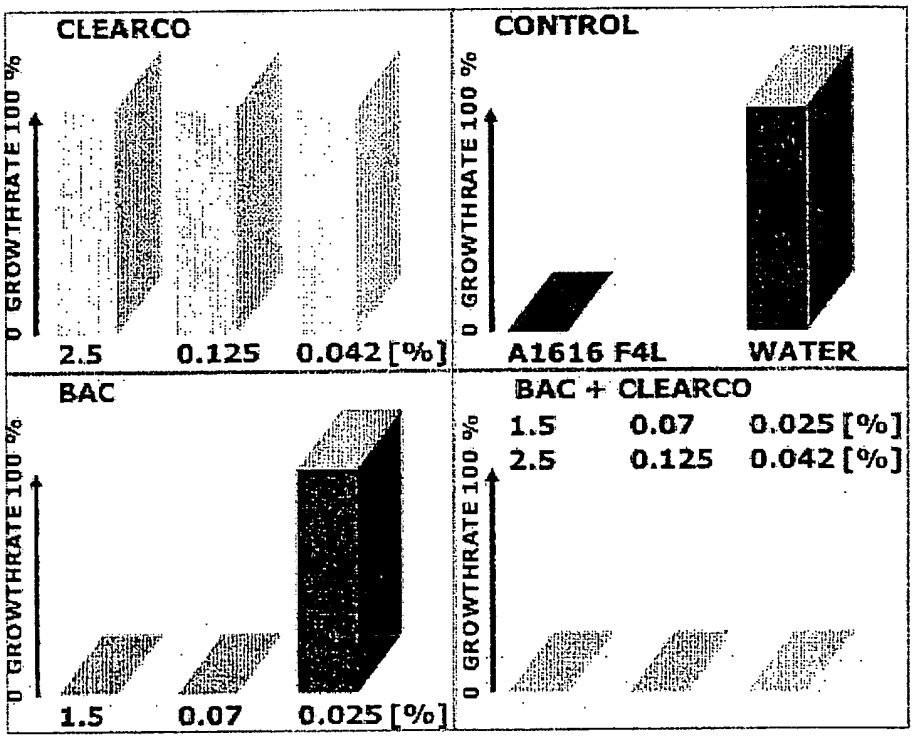
Evaluation

The plates were then evaluated for viable colony forming units (CFUs) such that 100% corresponds to an uncountable number of CFUs and 10% corresponds to approximately 10^2 CFUs. The results are presented graphically below.

Results and Interpretation

As shown in the graphs below, under the test conditions described above BAC alone was active as an anti-microbial agent at concentrations of 1.5 % and 0.07 % but it was ineffective at a concentration of 0.025 %. The polymer CLEARCO showed no antimicrobial activity when used alone. The combination of BAC and CLEARCO

showed strong antimicrobial activity at all the concentrations tested. Particularly significant is the fact that the combination of BAC and Clearco having a BAC concentration of 0.025% had much stronger antimicrobial activity that this concentration of BAC in the absence of the low surface tension material Clearco. The general activity of the test design was cross checked by using water as control 1 (no antimicrobial activity) and the Caddycare (strong antimicrobial activity) as control 2.



Testing "additive effect" of different polymers at limited biocide concentrations: Pretreatment Of Agar Plates

Testing of "additive effect" was done at limited (very low) biocide concentrations and combinations of the biocide (BAC) and the low surface tension materials CLEARCO, PS034, and ACTIVE SILICONE were used.

MATERIALS

LB Agar plates, LB Medium, BAC, CLEARCO, PS034, and ACTIVE SILICONE, Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium (OD_{600}: 4.3 / mL (Reference: LB Medium) ≙ 4.3 x 10^9 cells/mL), deionised water.

Test liquids

BAC	CLEARCO	PS034	SILICONE
0 %			
0.019 %			
0.015 %	0.031 % plus 0.93 % Ethanol	0.031 % plus 0.93 % Ethanol	0.031 %
0.0125 %			
0.011 %			

Compositions comprising each of the polymers and BAC at each of the concentrations shown above were prepared.

TESTING PROCEDURES

Pre-treatment of agar plates
1 mL of the test solutions (as shown above) was added to LB Agar plates and spread of the surface of the plates as a film.

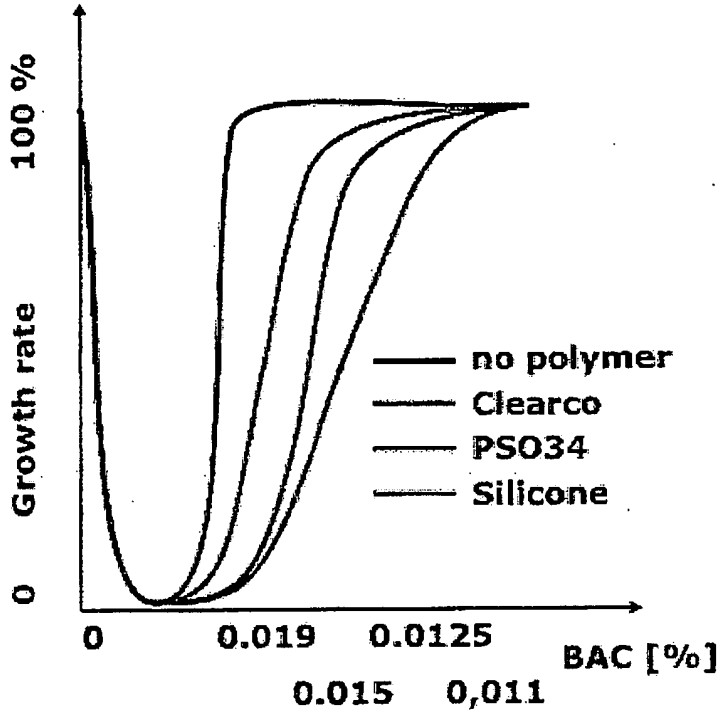
Challenge with E. coli
Each pre-treated LB Agar plate was challenged with 300 µL E. coli (10^6 cells in LB Medium) covering the agar surface.

Incubation
The Agar plates were incubated at 37 °C for 48 hrs.

Evaluation
The plates were then evaluated for viable colony forming units (CFUs) such that 100% corresponds to an uncountable number of CFUs and 10% corresponds to approximately 10^2 CFUs. The results are presented graphically below.

Results and Interpretation

As shown in the graph below, all of the polymers used significantly enhanced the efficacy of the BAC in compositions containing a low concentration of BAC compared to the same concentration of BAC in the absence of a polymer.



ANNEX II

REPORT OF EXPERIMENTS CONDUCTED TO ILLUSTRATE THE RESIDUAL EFFECT OF THE COMPOSITIONS WHICH ARE THE SUBJECT OF US PATENT APPLICATION NO. 10/039,677

Experiments were conducted to show the residual effect of the compositions that are the subject of US patent application no. 10/039677.

EXPERIMENT SET I

The antimicrobial formulation Marquat MQ624M was used. This formulation comprises:

ACTIVE INGREDIENTS:	
Octyl Decyl Dimethyl Ammonium Chloride	3.0%
Didecyl Dimethyl Ammonium Chloride	1.5%
Dioctyl Dimethyl Ammonium Chloride	1.5%
Alkyl (C_{14}, 50%; C_{12}, 40%; C_{16}, 10%) dimethyl benzyl ammonium chloride	4.0%
INERT INGREDIENTS:	90.0%
TOTAL	100.0%

This material was used neat and was also used in mixture with 1% and 5 % by weight of the Marquat MQ624M of a polydimethylsiloxane polymer (obtained from Fluorochem as Fluorochem 034).

As used hereinafter, the neat formulation (Marquat MQ624M) will be referred to as formulation A, the formulation comprising 1 % by weight of the polysiloxane will be referred to as formulation B and the formulation comprising 5 % by weight of the polysiloxane will be referred to as formulation C.

Formulation A is a control in that it does not contain the polymer, which is an essential feature of the compositions of the invention which is the subject of US Patent Application No. 10/039677. Formulations B and C are illustrative examples of compositions of the invention containing different concentrations of the polymer.

Experiment 1

A polyethylene Petri-dish was treated with 1 mL of formulation A and the surface was allowed to dry. When the surface was completely dry 1 mL of protein solution (about 3 % casein peptone mixture in liquid broth) was applied. The applied mixture was

dried at about 20 °C for at least one hour. This dried material was used to simulate the *extra*-cellular secretions of certain bacteria that constitute the "adherant" part of the biofilm formed by those bacteria at a surface.

The surface was then rinsed extensively with distilled water.

The rinsing step did not remove the protein layer or the layer of formulation A.

Experiment 2

The procedure of Experiment 1 was repeated except that 1 mL of formulation B was used instead of formulation A.

The rinsing step removed most of the protein layer but did not remove the layer of formulation B.

Experiment 3

The procedure of Experiment 1 was repeated except that 1 mL of formulation C was used instead of formulation A.

The rinsing step removed nearly all of the protein layer but did not remove the layer of formulation C.

Comments

As described in Experiment 1 above, the protein layer used in each of experiments 1 to 3 was considered to simulate the biofilm of a microbial colony.

The results obtained in Experiment 1 indicate that a biofilm would be able to adhere is a surface treated with the antimicrobial formulation A (ie neat Marquat MQ624M). The results obtained in Experiments 2 and 3 indicate that a biofilm would be unable to adhere to a surface treated with an anti-microbial composition of the invention containing a polydimethylsiloxane (formulations B and C).

As indicated at page 1, lines 22 and 23 of the application, adhesion to a surface is an essential step in biofilm formation. Thus, the results obtained show that the presence of a polydimethylsiloxane in an antimicrobial composition will significantly reduce the ability of a microbial colony to form on a treated surface. This results in a residual effect, ie not only do the compositions of the invention kill microbes present at a surface at the time a surface is treated with the composition, they also prevent further biofilm formation at the surface to which they have been applied. This effect could not have been predicted from either of the documents cited by the examiner.

Further experiments were undertaken to demonstrate that the compositions of the invention continue to exhibit anti-microbial properties at a surface to which they have been applied in addition to suppressing biofilm formation as illustrated above.

EXPERIMENT SET II

Experiment 4

500 µL of E. coli solution that approximated to 10^5 colony forming units was applied to each of 6 HDPE (High Density Polyethylene) Petri dishes which had each been treated with different concentrations of the following formulation (F4L) as indicated in the Table below:

F4L

Composition	%
Coco alkyl dimethylbenzyl ammonium chloride	20.000%
di-n-decyl dimethylammonium chloride	20.000%
bronopol (INN)	20.000%
Polymeric Biguanide Hydrochloride	20.000%
water	0.000%
ethanol	18.000%
Poly-dimethyl siloxane	2.000%
Totals:	100.00%

Formulation F4L is a composition of the invention as defined in patent application no. 10/039677.

Petri-dish		Concentration of F4L
A	Control	Distilled water only
B	Neat	No added water (100% Conc)
C	1:4	1 part F4L: 4 parts Water (20% Conc)
D	1:9	1 part F4L : 9 parts water (10% Conc)
E	1:19	1 part F4L : 19 Parts water (5% Conc)
F	1:39	1 part F4L : 39 parts water (2.5% Conc)
G	1:79	1 part F4L : 79 parts water (1.25% Conc)

15 minutes after the applied solution had completely dried 500 µL of liquid broth were used to dissolve E. coli. From each of the Petri dishes. 300 µL of the solution obtained from each Petri dish was applied to a separate LB agar plate. The LB agar plates were then incubated for 24 hrs at 37 °C. Standard methods were then used to determine the presence of E. coli on the LB agar plates.

There were no visible colonies present on any of the LB agar plates obtained from Petri dishes, which had been treated with the F4L formulation at any dilution. However, on the LB plate obtained from the control Petri dish (A) the number of E. coli colonies was too numerous to count.

Comments

The results of this experiment show that the compositions of the invention when present at a surface kill microbes that contact that surface subsequent to application of a composition of the invention to the surface even at dilutions as much as 1:79 (F4L:deionizeed water).

Conclusions

The experiments reported above illustrate that the compositions that are the subject of US patent application no. 10/039677 have a residual effect in that they prevent biofilm formation at a surface to which they have been applied and they also kill micorobes that contact such a surface subsequent to that surface being treated with a composition of the invention.